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Tubular and endothelial chimerism in renal allografts using fluorescence and chromogenic in situ hybridization (FISH, CISH) technology

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ABSTRACT

The role of endothelial- and tubular-chimerism in renal-allograft-adaptation and rejection varies in different studies. We addressed the correlation between different clinico-pathological-settings and sex-chromosomal endothelial- and /or tubular-chimerism in renal-allografts.

We examined the presence or absence of the X and Y-chromosomes by fluorescence and chromogenic-in-situ-hybridization (FISH, CISH) methodology on paraffin-embedded-kidney-biopsies in 16 gender-mismatched-renal-transplants (1 to 12-years post-transplantation). Twelve patients were male, 4 female. Four groups were selected: 1) Vascular calcineurin-inhibitor-toxicity without rejection, 2) T-cell-mediated-vascular-rejection, 3) antibody-mediated-rejection and 4) C4d-positivity in AB0-incompatible-transplants with or without rejection. Twelve non-transplant-kidney-biopsies (8-females, 4-males) were controls.

Tubular-chimerism was detected more frequently (69%) than endothelial-chimerism (12%) in renal-transplants. One of 12 control-patients had tubular and endothelial-chimeric cells (8%).

The Y-chromosome occurred in 8/12 male-recipients (67%) in tubular-epithelial-cells and in 5/12 male-recipients (42%) in endothelial-cells. Double X-chromosomes were detected in 3/4 female-recipients in tubular-epithelium.

Tubular-chimerism occurred more often with endothelial-chimerism and capillaritis without correlation with other parameters as rejection. Combined -Y-chromosomal tubular and lymphatic-endothelial-chimerism correlated with T-cell -mediated vascular-rejection in 2 of 3 patients (66%). Combined Y-chromosomal - tubular and peritubular-capillary chimerism correlated with antibody-mediated -C4d+ rejection in 1 of 2 patients (50%).

Tubular and/or endothelial-chimerism occur frequently in gender-mismatched-renal-allografts and when combined, this is associated with T-cell-mediated-rejection.

Keywords: endothelial and tubular chimerism, renal allografts, X and Y chromosome

INTRODUCTION

The role of chimeric cells in graft adaptation has been subject of interest in transplantation research for a long time. Research data go back to the 1960s, when Medawar postulated endothelial replacement through recipients cells as a possible mechanism for graft adaptation ^{1, 2}. Only very few studies are available in the field of renal allografts since then asking the question whether graft failure and /or successful adaption can be connected to the presence or absence of chimeric cells ³⁻⁶. The exact role of chimeric cells and their association to graft failure could not be answered unequivocally in these studies ³⁻⁶.

For this purpose we addressed the frequency of recipient derived chimeric cells in long-term kidney allograft recipients in this study. Furthermore we investigated whether the presence of chimerisms can be related to signs of rejection. We created four different clinico-pathological groups of renal transplant biopsies with and without detection of donor specific antibodies (DSA), signs of T-cell mediated vascular rejection (TMR) and/or C4d positivity. We analyzed the X and Y chromosomes in tubular epithelial cells, in endothelial cells of the peritubular capillaries, in lymphatic vessels and in interlobular arteries of 16 renal allograft biopsies from gender-mismatched transplantations more than one year post-transplant. As a control population, we analyzed 12 cases of diagnostic non-transplant kidney biopsies from 8 female and 4 male patients.

MATERIALS AND METHODS

Patients study groups

Data on kidney transplants were retrieved from the archives of the Division of Nephrology, University Hospital Zurich, Switzerland from the period 1996-2008.

Sixteen gender mismatched renal transplant patients with clinical follow-up could be identified, for whom late biopsies (> 1 year post transplantation) were available. All kidney biopsies were analyzed at the Institute of Surgical Pathology, University Hospital Zurich.

The age of the recipients' varied from 20 to 64 years (median age: 49.5 years).

Twelve recipients were male and 4 were females. Between 1 and 12 years (median time: 4.81 years) elapsed from time of transplantation to the first allograft biopsy. No data was available as to whether any of the donors had received blood transfusion or if any of the female donors had given birth to a son.

Clinical data of the study groups

Patients were divided into 4 groups according to different clinical settings (Details in Table 1).

Group 1 consisted of renal transplants with histological and clinical signs of chronic calcineurin inhibitor nephrotoxicity (CNI arteriolopathy) without evidence of acute or chronic rejection. C4d was negative in these biopsies and no DSA was found.

Group 2 contained biopsies with histologically proven acute T-cell mediated vascular rejection (TMR), Banff grade IIA which were C4d negative and without proven DSA. Chronic vascular T- cell mediated rejection was present in two of these patients (Nr 6 and 8).

Group 3 included patients with proven DSA, evidence of C4d deposition in peritubular capillaries on immunofluorescence examination and histological signs of combined acute TMR, Banff grade IIA and acute (Nr. 9) or chronic active (Nr.

10,11,12) antibody mediated rejection (AMR). In three patients (Nr. 9,11,12) a combined rejection (AMR and TMR) was present.

Group 4 consisted of patients with either normal histological and /or clinical findings or with acute vascular T-cell mediated rejection (1 or 2 years post transplantation in protocol biopsies). C4d was diffusely positive in these biopsies due to ABO incompatible transplantation and no DSA were found. DSA specific anti HLA antibodies were negative.

Banff classification was used for the histological criteria of rejection ⁷.

Patients and clinical data in the control groups

Diagnostic kidney biopsies from 12 non-transplant patients were selected as control tissue. Eight patients were female and 4 patients were male. The age of the patients varied from 13 to 89 years (median age 46 years). Four female patients were diagnosed with lupus nephritis, 2 with focal segmental glomerulosclerosis (FSGS), one with extracapillary proliferative glomerulonephritis (ANCA associated) and one with thin basement membrane disease. Two male patients were diagnosed with IgA nephropathy, and two with membranous glomerulonephritis (details in Table 1).

Histology

Kidney biopsies were fixed in 4% neutral buffered formalin, embedded in paraffin and sectioned according to the standard processing of transplant kidney biopsies at the Institute of Surgical Pathology: Up to 24 serial sections were cut from a paraffin block, and half of them were stained with the conventional stains: H&E (Hematoxylin & Eosin), PAS (Periodic-Acid Schiff), Ag (Silver-Methenamine), EVG (Elastin Van Gieson), and SFOG (Acid Fuchsin Orange G). The remaining slides were kept

unstained. All slides were re-evaluated by renal pathologists (ZV and AG) on each biopsy, and the diagnoses listed above were re-confirmed.

Data on C4d positivity (performed routinely on frozen kidney biopsies) was available in the original histology reports.

For the study, new slides of serial sections 2 μ m thickness were cut from the paraffin blocks.

Immunohistochemistry

D2-40 and CD31: These reactions were carried out on each graft biopsy in order to visualize lymphatic endothelial cells, peritubular capillaries, arterioles and interlobular arteries. Following primary antibodies and dilutions were used: D2-40 (DAKO A/S Glostrup, Denmark, M3619, dilution 1:50) and CD31 (DAKO, A/S, Glostrup, Denmark, IR610/IS610, dilution 1:50).

CD45 immunohistochemistry was performed on each graft biopsy to visualise lymphocytes using following antibody: CD45 (DAKO A/S Glostrup, Denmark, M0754, dilution 1:50).

CD3, CD4, CD8 and CD56 reactions were determined on four kidney biopsies with evidence of acute cellular rejection. Following antibodies were used: CD3 (Neomarkers, Fremont, CA, USA, RM-9107-S, dilution 1:100), CD4 (Ventana, Roche, Basel, Switzerland, 790-4423, prediluted), CD8 (DAKO, A/S, Glostrup, Denmark, M7103, dilution 1:100), CD56 (Novocastra, New Castle, UK, NCL-L-CD56-1B6, dilution 1:50).

All immunohistochemical reactions were performed with the Ventana Benchmark automated staining system using Ventana reagents for the entire procedure.

Reaction products were detected using the UltraVIEW DAB detection kit and the signal was enhanced with the amplification kit.

Fluorescence in situ hybridization (FISH) for chromosomes X and Y

Two types of direct fluorescent-labeled DNA probes were obtained from Pathvysion (Vysis, Abbott AG Diagnostic Division, Baar, Switzerland). Both reactions were performed using the VP2000 processor as recommended by the Vysis protocols. Briefly, probe mixes were hybridized at 37°C between 14 and 20h, washed in Rapid-Wash-Solution I at 73°C for 5min, Rapid-Wash-Solution II and H₂O for 7min, air dried and counterstained with 4',6-diamidino-2-phenylindole (DAPI).

Green and Orange signals were visualized by an Olympus microscope. Probes (30-161050 and 32-161050) labeled X-chromosome with orange color, Y-chromosome with green. The probes (AneuVysion) were labeled as follows: the X-chromosome with green and the Y-chromosome with orange.

The slides were all counterstained with DAPI.

FISH reactions were performed only on the graft biopsies.

Chromogenic in situ hybridization (CISH) for chromosomes X and Y

For this assay, a direct digoxigenin (DIG) and 2,4-dinitrophenyl (DNP) labeled in situ hybridization kit was used (CISH ZytoDot 2C, CEN X/Y probes, PD28, Zytovision, Labforce AG, Switzerland). The reactions were performed manually as recommended by the Zytovision protocols.

Briefly, the probe mixes were hybridized overnight at 37°C and washed in the same way as described by FISH above. The X chromosome was detected with an alkaline phosphatase (AP) labeled anti-DNP antibody and was visualized as a red spot, the Y

chromosome was detected with a horseradish peroxidase (HRP) labeled anti-DIG antibody and was visualized as a green spot. In order to visualize the morphology of the kidney tissue, the hybridized tissue was counterstained with haematoxylin for 2 minutes and eosin for 30 seconds, enabling precise morphology identification under a light microscope.

CISH signals were analyzed by conventional light microscopy.

CISH reactions were performed on all biopsies including the graft biopsies and the control cases.

Interpretation of FISH and CISH signals

The evaluation of chimeric cells was performed without knowledge of the clinical parameters.

The detection of the X and Y chromosomes was evaluated as positive, if the signals were identified within the nuclei of the tubular epithelium or inside lymphatic, capillary or arterial endothelial cells.

Specificity of FISH and CISH reactions was confirmed on normal kidney tissues from non transplant kidneys. Female phenotype was defined as two spots of the same color for X chromosome within one cell. Male phenotype was defined as two spots with two different colors within one cell (one color corresponded to Y chromosome, the other color to X chromosome).

The exclusion of recipient derived cells in the endothelial cells respectively in the tubular epithelial cells was done as follows: Serial sections were made and these were all controlled with H&E and corresponding immunohistochemistry for CD45, CD31 and D2-40.

By using the eosin nuclear stain for CISH, DAPI nuclear stain for FISH and controlling the site of the reaction on the adjacent H&E and immunohistochemistry, the anatomical localization of the signals (tubular epithelium, infiltrating lymphocytes lymphatic or capillary endothelial cells) was possible.

Finding at least one tubular, lymphatic or capillary endothelial cell containing two X chromosomes (from male donors) or one Y chromosome and one X chromosome (from female donors) were considered as a chimeric cell. Donor phenotype was defined as follows: two X chromosomes in a male recipient of a female graft or one Y and one X chromosome in a female recipient of a male graft. In the control group two X chromosomes in a female kidney or one X and one Y chromosome in a male kidney were considered as a normal finding.

X and Y chromosomal chimerism in the vessels was assessed only by CISH methodology. Tubular chimerism was evaluated with FISH and CISH methodology.

Scoring immunophenotype in interstitial and endothelial lymphocytes

Positivity scale: +: scattered cells positive, ++: < 50% of the cells positive, +++: > 50% of the cells positive.

Statistical analysis

The four groups and the presence or absence of chimeric cells in lymphatic, capillary, arterial endothelial cells or in tubular epithelial cells were statistically compared using the Fisher's exact test and the Chi-square test.

RESULTS

Normal tubular epithelium

Tubuli without pathological changes were present in all cases independently from the clinical setting. These tubuli contained either two X chromosomes in male recipients of a female graft or one Y and one X chromosome in female recipients of a male graft (Fig 1 A,B,D). In the control group tubuli displayed two X chromosomes in female kidneys or one X and one Y chromosome in male kidneys (Fig 1C).

Recipient derived lymphocytes

Recipient derived lymphocytes were present in most graft biopsies (Fig 2 A-D, Fig 3 A/C).

Chimeric cells in the study group (Table 2A).

X chromosomes in tubular epithelium

Three of 4 male grafts (75%) in female recipients had double X chromosomes.

X chromosomes in vessels

We could not identify any X chromosomal chimerism in vessels.

Y chromosomes in vessels

Lymphatic endothelial cells had Y chromosomes in 3 of 12 male recipients (25%).

Capillary endothelial cells had Y chromosomes in 2 of 12 male recipients of female grafts (17%). (Fig 3 D, Fig 4 A-D, Fig 5 A-D).

Y chromosome in tubular epithelium

Y chromosome chimerism was detected in 11 of 16 grafts (69%). Eight of 12 female grafts (67%) had scattered Y chromosomes (Fig 3 B). Double Y chromosomes were

seen in 2 of 4 male grafts (50%) in female recipients (Fig 6 A-D). Chimeric cells were mainly seen in the proximal tubules in a dispersed fashion intermixed with numerous epithelial cells of the graft. Most tubular epithelial cells were of donor origin and chimeric cells were less numerous.

Chimeric cells in the control group (Table 2B)

We could not identify chimeric cells in any of the eight female kidney biopsies (0/8). Among the male patients, 1 kidney (Nr. 28) had double X chromosomes in the tubular epithelium and double Y chromosomes in the endothelial cells in an interlobular artery (1/4, 25%, 1/12 8%).

D2-40 detection in lymphatic vessels and in peritubular capillaries

Lymphatic vessels were present in each kidney biopsy. Most of these were visualized in the peritubular regions. The density of these capillaries varied to some extent, however no significant differences were observed in the different groups.

CD31 detection in peritubular capillaries, arterioles and interlobular arteries.

Peritubular capillaries, arterioles and interlobular arteries were present in all kidney biopsies and were decorated by a CD31 endothelial reaction.

Immunophenotype of lymphocytes in graft biopsies

Interstitial and endothelial lymphocytes in the graft biopsies were positive for following marker: CD4 (+++), CD8 (+), CD3 (+++), CD56 (negative).

Comparison of FISH and CISH methodology in graft biopsies

In 4 of 16 cases, there was a discrepancy between FISH and CISH testing. In 3 male patients FISH detected Y chromosomes in tubular epithelium, in one male patient Y chromosomes were detected only with CISH testing. In the 4 discrepant cases, we arbitrarily included the results with CISH.

Correlation of chimerism to clinical and histological parameters

All grafts with endothelial chimerism also had tubular chimerism; thus we could not detect isolated endothelial chimerism without tubular chimeric cells. The detected association between tubular and endothelial location of chromosome chimerism could statistically only be demonstrated as a trend (Fisher's exact test: $p=0.119$) but did not reach significance.

Additionally, combined tubular and endothelial Y chromosome chimerism (Nr. 1, 6, 9, 11, 16) was associated with acute T cell mediated rejection in 3 of 5 grafts.

Tubular Y chromosome chimerism was found more often in association with capillaritis (4 out of 6 patients), which did not reach significance.

Tubular Y chromosome chimerism did not show any correlation to any further clinic-pathological parameters (Fischer's exact test: $p=0.464$).

An X chromosomal tubular chimerism could not be correlated to any of the clinocopathological factors. No X chromosomal endothelial chimerisms were found in our study cohort.

Chimeric cells, either with X or with Y chromosome abnormalities, were equally present in all four clinical subgroups (Chi-square test: $p=0.362$).

DISCUSSION

In this study we examined the frequency of X and Y chromosomal chimeric cells in allograft biopsies of gender-mismatched renal transplants. We were interested whether tubular and lymphatic or capillary endothelial cells displaying chimeric features could be related to TMR, AMR, C4d positivity in ABO incompatible grafts or CNI nephrotoxicity.

Our data show that sex chromosomal chimerism, particularly Y chromosome chimerism is a common finding in kidney allograft biopsies from gender-mismatched transplants occurring in approximately 40-70% of kidney biopsies in both genders more than 1 year post-transplant. We found that combined Y chromosomal chimerism in tubular and in endothelial cells (particularly in lymphatic endothelial cells) can be related to acute T cell mediated rejection. Tubular Y chromosomal chimerism alone could not be associated to any known clinical or morphological factor such as rejection or calcineurin nephrotoxicity and they were equally represented in all four subgroups (Chi-square test: $p=0.362$). However, in our series an endothelial chimerism was always accompanied by a tubular chimerism. Moreover, a tubular chimerism was more frequently associated with endothelial chimerism ($p=0.119$).

The association between endothelial replacement by recipient-derived cells and graft adaption was first described by Peter Medawar, who postulated a good prognosis of the graft if chimeric cells were present ^{1, 2}. Since his original description, this issue has been investigated in a few studies with divergent results. Lagaaij et al. detected a strong association between acute rejection and the number of endothelial chimeric cells in transplant kidney biopsies ⁴. In a study by van Poelgeest et al., all female recipients and half of the male recipients displayed blood group antigen chimerism in the endothelial cells, which showed a positive correlation to the degree of tubular

atrophy. However this correlation lacked any association to clinical features such as rejection or allograft survival⁶. Bai et al described a similar finding, they found that endothelial chimerisms in the arterioles were common and that they occurred regardless from rejection³. In an experimental rat model, a high degree of endothelial chimerism was found when the animals obtained ischemic injury and were treated with immunosuppressants⁸. In previous studies it was found that tubular chimerisms on the other hand seem to be a frequent finding in renal allografts occurring in up to 88% of the male patients receiving a female kidney^{5,9}.

The presence of lymphatic neogenesis in renal transplants and also an association of lymphatic cells to rejection have been described by Kerjaschki et al.¹⁰. In our series we showed the presence of lymphatic vessels in all transplant biopsies (irrespective of the rejection status), and we found an association between lymphatic endothelial Y chromosomal chimerism and acute T-cell mediated rejection. We furthermore showed that lymphatic endothelial Y chromosomal chimerisms do not occur without tubular Y chromosomal chimerisms. This combined phenomenon was seen in 75% of the cases in patients with T-cell mediated acute rejection. We did not see chimeric cells in the arterioles in our study.

In our study 42% and 67% of the male patients displayed recipient derived cell phenotype in endothelial and in tubular epithelial cells, respectively. Three of these patients had chimeric cells in lymphatic endothelial cells, 2 of them in the peritubular capillaries. As to X chromosomal chimerism, we could detect only X chromosomal tubular chimerisms in 3 of 4 female recipients and no X chromosomal chimerism were found in the endothelial cells in our cohort. We were not able to correlate these findings to any clinical or pathological parameters especially to rejection or C4d positivity as evidence for antibody deposition and complement activation.

Sex chromosomal chimerisms can frequently be found in patients with systemic lupus erythematoses ^{11, 12}. In a study Kremer Hovinga et al. described that sex chromosomal chimeric cells in kidney biopsies were found twice as often among women who were suffering from the lupus disease than those who were free of this disease ^{11, 12}. We investigated 8 diagnostic kidney biopsies from women, 4 of which had lupus nephritis. We failed to detect any chimeric cells in any of the examined structures in any of the female patients. Surprisingly, one of the four male control patients (Nr. 28) had chimeric cells in tubular epithelium and in the endothelial cells in an interlobular artery, even though this patient did not suffer from lupus nephritis but was diagnosed with IgA nephropathy. The clinical impact of chimeric cells in this patient was probably not relevant, as he did not develop proteinuria. Double aneuploidy involving both sex chromosomes, as was detected at this patient (Nr. 28) can occasionally occur in otherwise healthy individuals ¹³. Some studies suggest that at least in a subset of affected individuals with sex chromosomal aneuploidy, maternal and paternal age (over 35) can play an ethiological role ¹³. Interestingly, in previous reports, chimeric cells were found in women, both, with and without previous blood transfusions and with or without giving birth to a son, respectively ^{11, 12, 14}. A similar finding was detected by Koopmans et al., who found chimeric cells in normal kidneys, liver and heart tissue received from autopsies from otherwise healthy women with a known history of blood transfusion or child birth ^{15, 16}. The presence of male chimeric cells in female recipients with or without children suggests that chimeric cells are not necessarily donor-derived and could have 'passed' into the organs prior to the transplantation ^{15, 16}. Other sources for chimeric cells prior to transplantation as fetal-maternal bidirectional cell trafficking and sexual intercourse have also been discussed in the literature ¹⁷. Kidney biopsies prior to

transplantation are helpful in ruling out preexisting chimeric cells. Unfortunately such biopsies were not available in our series.

Poulsom et al. and Terada et al. have described evidence, that organ specific stem cell populations are capable of adopting the phenotype of the recipient cells by cell fusion^{18, 19}. According to their findings, the presence of bone marrow derived cells with recipient analogous phenotype can contribute to normal turnover of renal epithelial cell regeneration after injury¹⁸⁻²⁰. Cell fusion seems to be the predominant mechanism in animal models in generating myocytes from bone marrow derivatives²¹. It is tempting to speculate that the high frequency of chimerisms in human renal transplants, which was also the case in our study, may derive from cell fusion. Another theory from Lin et al, suggests that surviving mature epithelial cells after renal injury are capable of dedifferentiating, proliferating and redifferentiating in order to restore tubular structures and function²². Both theories provide a possible explanation for the high percentage of recipient derived Y chromosomal chimerisms (over 70%) after kidney transplantation in our study.

Y chromosomal chimerism has been described to occur at a higher frequency in tubular epithelium than the X chromosome^{9, 23}. In these studies, Y chromosomal tubular chimerisms can be detected in up to 70% of the mismatched grafts, which is very similar to our findings^{9, 23}.

The presence of recipient derived mesenchymal cells of the vessel-intima was described in a study by Grimm et al., nevertheless converse to our results, Grimm et al. could correlate these data to chronic rejection²⁴.

Further details for chimerisms include theories such as graft-versus-host reaction, host-versus-graft reaction as well as a beneficial function of chimeric cells that may play a role in repair mechanisms¹¹. Women, who gave birth to a son, could 'develop'

chimeric cells by incorporating fetal cells into their own circulation, resulting in a so-called fetal chimerism ¹¹. On the other hand, maternal chimerism can be a possible explanation for neonatal lupus erythematosus or similar entities such as juvenile inflammatory myopathies or dermatomyositis through circulating maternal cells into the offspring ^{11, 14}.

In summary, we could show that Y chromosomal chimerism is a common finding in gender mismatched renal allografts. In combination with lymphatic endothelial chimerism, Y chromosomal tubular chimerism can be associated to T cell mediated acute rejection but not to C4d positivity and/or antibody mediated acute rejection. The exact biological significance of sex chromosomal chimerism in renal allograft dysfunction however still remains controversial and needs further studies to clarify its role.

LEGEND OF FIGURES

Figure 1

(A): Normal tubular epithelium contains 2 X chromosomes in a male recipient from a female transplant kidney. FISH reaction (X chromosome: orange).

(B): Normal tubular epithelium with 1 X chromosome and 1 Y chromosome in a female recipient from a male transplant kidney. FISH reaction (X chromosome: green; Y chromosome: orange).

(C): Normal tubular epithelium contains 2 X chromosomes in a non transplant female kidney. CISH reaction (X chromosome: red).

(D): Normal tubular epithelium with 1 X chromosome and 1 Y chromosome in a female recipient from a male transplant kidney. CISH reaction (X chromosome: red; Y chromosome: green).

Figure 2

(A): Tubular epithelium shows tubulitis from a CD45 positive recipient derived lymphocytes in a male recipient in a female transplant kidney.

Immunohistochemistry for CD45.

(B): Glomerular capillary loops with glomerulitis from a CD45 positive recipient derived lymphocytes of a male recipient in a female transplant kidney.

Immunohistochemistry for CD45.

(C): Tubular epithelium with tubulitis with recipient derived lymphocytes (arrows) of a male recipient in a female transplant kidney. CISH reaction (Y chromosome: green; X chromosome: red). This illustration is a serial section to Fig 2A.

(D): Glomerular capillary loops with recipient derived lymphocytes (arrows) of a male recipient in a female transplant kidney. Note mesangial cells of the female donor containing two X chromosomes (arrowhead). CISH reaction (Y chromosome: green; X chromosome: red). This illustration is a serial section to Fig 2B.

Figure 3

(A): Tubular epithelium without tubulitis. Scattered CD45 positive recipient derived lymphocytes (arrow) are outside the tubulus in a male recipient in a female transplant kidney. Immunohistochemistry for CD45.

(B): Tubular epithelium without tubulitis. One tubular epithelial cell shows 1 Y and 1 X chromosome (arrow), the other tubular epithelial cells derive from the donor (2 X chromosomes). Male recipient in a female transplant kidney.

CISH reaction (Y chromosome: green; X chromosome: red).

(C): Peritubular capillary without endothelitis. Scattered CD45 positive recipient derived lymphocytes are not in the endothelial cells (arrow). Male recipient in a female transplant kidney. Immunohistochemistry for CD45.

(D): Peritubular capillary (PTC) without endothelitis. Endothelial cells with one Y chromosome (arrow). Male recipient in a female transplant kidney. CISH reaction (Y chromosome: green).

Figure 4

(A): Peritubular lymphatic vessels stained positively (arrow). Peritubular capillaries and an interlobular artery are negative. Immunohistochemistry for D2-40.

(B): Endothelial cells in a peritubular lymphatic vessel contain 1 Y and 1 X chromosome (arrow). All other cells are donor derived (2 X chromosomes). Male recipient of a female kidney. CISH reaction (Y chromosome: green; X chromosome: red).

(C): Endothelial cells in a peritubular lymphatic vessel are without endothelitis. Negativity for CD45 in the endothelial cells (arrow), only interstitial recipient derived lymphocytes are positive. Immunohistochemistry for CD45.

(D): Endothelial cells in a peritubular lymphatic vessel with one Y chromosome (arrow). Male recipient of a female kidney. FISH reaction (Y chromosome: orange).

Figure 5

(A): Endothelial cells of an interlobular artery are stained positively for CD31 (arrow). Peritubular capillaries and lymphatic vessels are negative. Immunohistochemistry for CD31.

(B): Endothelial cells in an interlobular artery contain 1 Y and 1 X chromosome (arrow). Male recipient of a female kidney. CISH reaction (Y chromosome: green; X chromosome: red (weaker signal)).

(C): Endothelial cells in an interlobular artery are without endothelitis. Negativity for CD45 in the endothelial cells (arrows) and only scattered interstitial recipient derived lymphocytes are positive. Immunohistochemistry for CD45.

(D): Endothelial cells in a peritubular lymphatic vessel with 1 Y and 1 X chromosome (arrow). Male recipient in a female transplant kidney. FISH reaction (Y chromosome: orange; X chromosome: green).

Figure 6

(A) Tubular epithelial cells showing 2 Y chromosomes (arrow) in a female recipient of a male kidney. All other cells are of the donor type showing 1 X and 1 Y chromosome. CISH reaction (Y chromosome: green; X chromosome: red).

(B) Higher magnification of the epithelial cells with 2 Y chromosomes from the same area (arrow). CISH reaction (Y chromosome: green; X chromosome: red).

(C) Tubular epithelial cells showing 2 Y chromosomes (arrow) in a female recipient of a male kidney. All other cells are of the donor type showing 1 X and 1 Y chromosome. FISH reaction (Y chromosome: orange; X chromosome: green).

(D) Higher magnification of the epithelial cells with 2 Y chromosomes from the same area (arrow). FISH reaction (Y chromosome: orange, X chromosome: green).

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